

(Item 6) 635 Barnhill Drive Indianapolis, IN

William E. Farquhar 855-3962 812 12c. NAME AND TITLE OF OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Item 15) Wendell F. McBurney 317 274-8285

13. USE THIS SPACE FOR CORRECTIONS TO ITEMS 1 THROUGH 6. INDICATE THE NUMBER(S) WHERE ANSWERS APPLY.

14. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense. (U.S. Code, Title 18, Section 1001.)

SIGNATURE OF PERSON NAMED IN 2a (In ink. "Per" signature not acceptable)

Hourste swon

DATE DATE

15. CERTIFICATION AND ACCEPTANCE: I certify that the statements herein are true and complete to the best of my knowledge, and accept the obligation to comply with the Public Health Service terms and conditions if a grant is awarded as the result of this application. A willfully faise certification is a criminal offense. (U.S. Code, Title 18, Section 1001.)

SIGNATURE OF PERSON NAMED IN 12c (In ink. Per" signature not acceptable)

KWON00056

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	ant organization only)				EFFORT ON		FRINGE	
NAME	ROLE IN PRO	OJECT	TYPE APPT.	% OF APPT.	PROJ.	SALARY	BENEFITS	TOTA
Byoung S. Kwo			1.0	46	0.46	28,130	9,522	37,65
Karen Z. Poll	ok Fellow	al	1.0	100	1.0	20,000	4,980	24,98
<u>Hal E. Broxme</u>	yer Co-Investi	gator	1.0	3	0.03	2,388	808	3,19
Kwi O. Oh	Co-Investi	gator	1.0	100	1.0	13,532	3,370	16,90
Yvonne Kobaya	shi Research T	ech.	1.0	100	1.0	20,168	5,023	25,19
Zhen Zhou	Visiting Scientist		1.0	30	0.3	4,400	1,096	5,49
	SUBTOTA	N.C				88.618	24,799	113,41
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GRANT NUMBER

SECTION I (continued) NEXT BUDGET PERIOD

AI 28175-02

B. SUPPLEMENTAL INFORMATION REGARDING *ITEMS* IN THE PROPOSED BUDGET FOR THE NEXT PERIOD WHICH REQUIRE EXPLANATION OR JUSTIFICATION. (SEE INSTRUCTIONS)

Personnel

Dr. Byoung S. Kwon, Principal Investigator (46% effort), is responsible for the overall conduct of this research. His efforts include performance of daily work at the laboratory bench; coordination of work with the collaborating laboratories of Drs. Hal Broxmeyer, Leonard Schultz, Sherman Weissman, and John Ding-E Young; administration of the project budget, and writing of annual reports. Dr. Kwon will design and construct expression plasmids, and perform receptor-binding studies and functional assays, evaluating each experimental step in the program.

Dr. Karen Pollok, (100% effort) is a Postdoctoral fellow in this laboratory. Her responsibilities will include protein purification of soluble 4-1BB and perforin and a variety of immunological assays. Dr. Pollok will perform a number of biochemical and receptor-binding assays to characterize 4-1BB molecule.

<u>Dr. Hal Broxmeyer</u>, Coinvestigator (3% effort), participates in evaluating functions of recombinant products of L2G25B and L2G25C in both in vitro and in vivo.

Dr. Kwi-Ok Oh, Coinvestigator (100%), a Visiting Scientist, has been receiving training in this laboratory since. She will maintain cell culture, transfect recombinant DNA and perform protein purification and receptor-binding assays L2G25B and L2G25C proteins under the instruction of Dr. Kwon.

Miss Yvonne Kobayashi, (100%) Research Technician, will prepare DNAs, perform Southern, Northern, and Western blotting procedures, and screen hybridomas by dotimmunoassay. Yvonne is well-trained in DNA-sequencing. She is also well trained in handling vaculovirus expression systems. She has been producing recombinant proteins for L2G25C, perforin and 4-1BB. Yvonne will also be involved in purification of a large quantity of perforin to crystalize.

 $\underline{\text{Dr. Zhen Zhou}}$, (30%) Visiting Scientist, will perform immunocytochemistry to localize the 4-1BB proteins in various mouse organs. He is also doing $\underline{\text{in}}$ $\underline{\text{situ}}$ hybridization with the gene probes we generated. Dr. Zhou will also be involved in the studies on 4-1BB and perforin expression in the T cells infiltrated in autoimmune insulitis and arthritis of mice.

Consultants \$600

The amount of \$600 is requested to defray travel expenses of Dr. Kwon for the purpose of conferring once each year with Drs. Weissman and Shultz. No cost-increase is projected in subsequent grant-years for this consultant expense.

<u>Dr. Sherman</u> Weissman will continue to provide his expertise in nucleic acid research in all phases of this project. He advises on production of recombinant proteins in both prokaryotic and eukaryotic expression systems. He also evaluates the work of this laboratory as to scientific merit.

<u>Dr. John D.-E Young</u> consults with Dr. Kwon on protein purification and assays on CTL activity.

<u>Dr. Leonard Shultz</u>, of Jackson Laboratories, provides immunodeficient mutants collected by him and also provides new mutants as they arise.

 $\underline{\text{Dr. Stephen Litwin}}$ consults with this laboratory on B-cell maturation, immunoglobulin production, and assays on B-cell functions.

<u>Dr. Himadri Samanta</u> provides his expertise on the production of recombinant DNAs using the bovine papilloma viral expression system. He also advises on the technologies for purification of the recombinant proteins.

KWON00058

Supplies

\$23,280

Items in the supply budget remain the same as in grant-year 2, and a 6% increment is applied to allow for increases in costs.

Trave1

\$1,200

Dr. Kwon and Dr. Pollok plan to attend FASEB meeting.

Other Expenses

\$1,600

Costs of publication (page charges, artwork, etc.), computer costs, and mailing/long-distance telephone expenses remain the same as in year 2.

SECTION II	FROM	THROUGH	GRANT NUMBER	
CURRENT BUDGET PERIOD				
AND KEY PERSONNEL			AI 28175-02	

The following pertains to your CURRENT PHS budget. This information will be used in determining the amount of support for the NEXT budget period.

A. CURRENT BUDGET	TOTAL ESTIMATED EXPENDITURES AND OBLIGATIONS (1)	ESTIMATED UNOBLIGATED BALANCE (2)	EXPLAIN ANY SIGNIFICANT ESTIMATED UNOBLIGATED BALANCE IN COLUMN 2 (3)
TOTAL DIRECT COSTS	99,493	99,493	
INDIRECT COSTS (As provided)	48,105	48,105	
TOTALS>	147,598	147,598	

B. CURRENT BUDGET PERIOD KEY PERSONNEL ENGAGED ON PROJECT (Only if different)

NAME, DEGREE(S) SSN	POSITION TITLE AND ROLE IN PROJECT DEPARTMENT AND ORGANIZATION	CHANGE IN % OF EFFORT
Karen Z. Pollok, Ph.D. 231-84-3958	Postdoctoral Fellow	+ 1.0
Yvonne Kobayashi, B.S. 306-64-6558	Lab Technician	+ 1.0
Zhen Zhou, M.D.	Visiting Scientist	+ 0.3

C. and D. (Only if different)

See instructions and provide the information required in Items C. and D. Use this page and continuation pages as necessary.

See Attached

SECTION III. PROPOSED KEY PERSONNEL FOR THE NEXT BUDGET PERIOD (Only if different)

NAME, DEGREE(S), SSN	POSITION TITLE AND ROLE IN PROJECT	DEPARTMENT AND ORGANIZATION
Karen Z. Pollok, Ph.D.	Postdoctoral Fellow	Microbiology and Immunology Indiana University School of Medicine
Yvonne Kobayashi, B.S.	Lab Technician	Microbiology and Immunology Indiana University School of Medicine
Zhen Zhou, M.D.	Visiting Scientist	Microbiology and Immunology Indiana University School of Medicine
		kwon00060

C and D (continued)

- C. Equipment Other funds were identified to purchase the requested Micro centrifuge. No other equipment was purchased.
- D. Travel The following trips were supported:
 - 1) B.S. Kwon to Berlin, W. Germany 7th International Congress of Immunology \$1,242 Presented a poster.
 - 2) B.S. Kwon New Orleans, LA \$727
 ASBMB/AAI Joint Meeting Spoke at a mini-symposium and presented a poster.
 - 3) Kack Kim Hilton Head, SC \$460
 Second International Workshop on cytokines.

 Presented a poster. Dr. Kim's salary is not supported by this grant but he is studying perforin gene expression, a part of this project. Dr. Kim is Dr. Oh's husband.
 - 4) Dr. Oh Hilton Head, SC \$257
 Second International Workshop on cytokines.

 Presented a poster.

OTHER SUPPORT

(Use continuation pages if necessary)

GRANT NUMBER

AI 28175-02

FOLLOW INSTRUCTIONS CAREFULLY. Incomplete, inaccurate, or ambiguous information about OTHER SUPPORT could lead to delays in the award. OTHER SUPPORT to be listed here refers to all current or requested support whether related to this application or not. If there are changes subsequent to submission, notify the Grants Management Official named on the Notice of Grant Award.

For each of the key personnel named on page 4, list, in three separate groups: (1) all currently active support; (2) all applications and proposals pending review or funding; and (3) applications and proposals planned or being prepared for submission. Include all Federal, non-Federal (e.g., for-profit, pharmaceutical, foundations), and institutional research, training, and other grant, contract, and fellowship support at the applicant organization and elsewhere. If part of a larger project, identify the principal investigator/program director and provide the data for both the parent project and the subproject. If none, state "none."

For each item give: (a) the source of support, identifying number and title; (b) percentage of appointment on the project; (c) dates of entire project period; (d) annual direct costs; (e) a brief description of the project; (f) whether the item overlaps, duplicates, or is being replaced or supplemented by the present application; delineate and justify the nature and extent of any scientific and/or budgetary overlaps or boundaries; and (g) any modifications that will be made should this continuation award be made.

- (1) CURRENTLY ACTIVE SUPPORT: (a)
 - a) NIH Grant 1R01 AR40248-01; Melanin Biosynthesis and Oculocutaneous Albinism
 - b) B.S. Kwon, Principal Investigator. 10% effort. c)
 - d) Annual cost \$128,705 e) Studies on the molecules involved in melanin pigment synthesis. f) Not overlap with current project.
 - a) Schering-Plough Corporation; Regulation of Melanin Biosynthesis (Chemicals)
 - b)B.S. Kwon, Principal Investigator. 10% effort. c)
 - d) Annual cost \$140,500 e) Induction of darkening and lightening of skin pigment by chemical agents. f)

 Not overlap with current project.
 - a) March of Dimes Birth Defects Foundation; Molecular Basis of tyrosinase-negative albinism b) B.S. Kwon, Principal Investigator. 5% effort. c)
 - d) Annual direct cost \$35,000 e) Molecular genetic studies on the mutation responsible for tyrosinase-negative albinism. f) Not overlap with current project.

KWON00062

SECTION IV	GRANT NUMBER		
PROGRESS REPORT SUMMARY	AI 28175-02		
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR	PERIOD CO	VERED BY THIS REPORT	
Byoung S. Kwon, Ph.D.	FROM	THROUGH	
APPLICANT ORGANIZATION	***************************************		
Indiana University School of Medicine			
TITLE OF PROJECT (Repeat title shown in item 1 on first page)			

Characterization of three new T lymphocyte-specific gene (SEE INSTRUCTIONS)

1. The main objective for the forthcoming project year is to determine the functions of L2G25B, L2G25C (formerly called L2G95B) and 4-1BB. We will also study the domain-specific function of perforin. We first prepared antibodies against oligopeptides representing the deduced primary sequence of each molecule and then prepared recombinant proteins of these molecules. The following is a brief summary of unpublished results and future plans.

A. 4-1BB:

4-1BB protein is a receptor molecule which is inducible in T cells and is expressed constitutively in neurons and in certain medulla cells in kidney. Cytoplasmic domain of 4-1BB contains consensus sequence (-C-R-C-P-) for the binding to p561ck, a protein tyrosine kinase. We are preparing soluble form of the 4-1BB, which is recombinant protein without the transmembrane domain. This can be a specific inhibitor to the 4-1BB, as shown in other lymphokine receptors. We have expressed 4-1BB in C127 cells using bovine papilloma viral vector and in insect cells (sf-9) using baculoviral vector. These material will be used for two purposes; 1) to find the potential ligand; and 2) to determine whether 4-1BB transduces signal through binding to protein tyrosine kinases such as p561ck.

B. Perforin:

Perforin is believed to be a key molecule in killing cells bearing foreign antigens by forming a transmembrane channel. We isolated and characterized the cDNA and a gene for mouse perforin. We have been successful in producing the recombinant perforin (both human and mouse) in a baculoviral expression system. The purpose is to crystalize the molecule to understand its three dimensional structure. This information in turn will be used to design experiment to determine the domain-specific functions of this molecule.

C. L2G25B and L2G25C:

We have prepared a large quantity of recombinant proteins for both genes. According to in vitro experiments done in collaboration with Dr. Hal Broxmeyer the L2G25B and L2G25C recombinant proteins have direct myelopoietic enhancing activity for mature progenitors, while L2G25B, but not L2G25C, has direct suppressing activity for more immature progenitors. We plan to determine the physiological relevance of the myelopoietic enhancing and suppressing activities of these molecules in animal models. We are also seeking cells bearing receptors to the L2G25B and L2G25C proteins. The cells that we are employing at present are FDCP-Mix, 416B, and DU528, which represent bone marrow stem cell lines; Il-l-activated endothelial cells; activated spleen cells; and primary bone marrow cell mixture. We hope this study leads to finding the target cells through which we can study in depth the functions of these small molecules.

- 2. Our main effort was directed to prepare reliable reagents to utilize for the determination of functions of T-cell molecules, 4-1BB, L2G25B, L2G25C and perforin. All the molecules have been produced in recombinant form and antibodies recognizing the natural molecules are prepared. In addition to the preparative work mentioned above, we found followings in each molecule. 1) 4-1BB was expressed not only in T-cells but also in neuron and kidney cells. 4-1BB was expressed in early stage of insulitis but not in later stage of insulitis in NOD mice. 2) 5' UTR (untranslated region) of mouse perforin is formed by alternative splicing. The perforin mRNA was degraded within 5 min. when the killer cells (CTL, NK and LAK) made a full contact with specific target. Mouse perforin gene was characterized and mapped to chromosome #10. 3) L2G25B and L2G25C recombinant proteins have direct myelopoietic enhancing activity for mature progenitors, while L2G25B, but not L2G25C, has direct suppressing activity for more immature progenitors. Our recombinant proteins for L2G25B and L2G25C were as active as natural ones.
- 3. and 4. Non-applicable.
- 5. Seven papers have been published or are now in-press and one is in submission during the second grant-year: Copies of those papers are attached.
- Kwon, B.S., Kestler, D.P., Eshhar, Z., Oh, K.-O. and Wakulchik, M. Expression characteristics of two potential T cell mediator genes. Cellular Immunology 121: 414-422, 1989.
- Qureshi, M., Yoon, J.W. and Kwon, B.S. Identification and production of monoclonal antibodies against a discriminating protein molecule between B and D variants of encephalomyocarditis virus. Develop. Comp. Immunol. 13: 263-271, 1989.
- Liu, C.C., Joag, S.V., Kwon, B.S., and Young, J.D-E. Induction of perforin and serine esterases in murine cytotoxic T lymphocyte clone. <u>J. Immunol</u>. 144: 1196-1201, 1990.
- Joag, S.V., Liu, C.C., Kwon, B.S., Duke, R.C., Clark, W.R., and Young, J.D.-E. The expression of pore-forming protein and two serine esterase genes in murine primary and cloned effector lymphocytes. <u>J. Cell. Biochem</u>. 43: 81-88, 1990.
- Trapani, J.A., Kwon, B.S., Kozak, C.A., Chintamaneni, C., Young, J. D-Z and Dupont, B. Genomic organization of the mouse pore-forming protein (perforin) gene and localization to chromosome 10. \underline{J} . $\underline{\text{Exp}}$. $\underline{\text{Med}}$. 171:545-557, 1990.
- Young, J.D.-E., Kwon, B.S., Trapani, J.A., Liu, C.-C. and Young, L.H. Lymphocyte-mediated cytolysis: role of granule mediators. <u>Subcellular Biochem</u>. (in press).
- Broxmeyer, H.E., Sherry, B., Cooper, S., Oh, K., Tekamp-Olson, P., Kwon, B.S., and Cerami, A. Enhancing and suppressing effects of recombinant murine macrophage inflammatory proteins on colony formation $\underline{\text{in vitro}}$ by bone marrow myeloid progenitor cells. $\underline{\text{Blood}}$. (in press).
- Bajapi, A., Kwon, B.S. and Brahmi, Z. Rapid loss of perforin and serine protease RNAs in cytotoxic lymphocytes exposed to sensitive targets. J. Immunol. (submitted)

		GRANT NUMBER
CHEC	KLIST	AI 28175-02
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ASSURANCES		
The following certifications described below SIGNING FOR APPLICANT ORGANIZATION	are made by checking the app I on the FACE PAGE of the ap	propriate boxes and verified by the signature of the OFFICIAL plication.
principal investigator/program director.	organization, <u>not</u> to the person	rtify that it is <u>not</u> delinquent on the repayment of any Federal debt n signing the application as the authorized representative <u>nor</u> to the
other miscellaneous administrative debts.	 For purposes of this certification 	s, guaranteed or direct student loans, FHA loans, business loans, action, the following definitions of "delinquency" apply:
For direct loans and fellowships (whet funds), a debt more than 31 days pass Service Award.)	ther awarded directly to the ap of due on a scheduled payment	oplicant by the Federal Government or by an institution using Federal. (Definition excludes "service" payback under a National Rese
For guaranteed and insured loans, recrepurchased from a lender because the	cipients of a loan guaranteed b	by the Federal Government that the Federal Government has agreement and is in default.
For grants, organizations in receipt of have not resolved the disallowance.	a "Notice of Grants Cost Disa (Definition excludes disallowar	allowance" which have not repaid the disallowed amount or which nces in an "appeal" status.)
Where the applicant discloses delinquence determining whether the prospective gran until payment is made or satisfactory arranger the PHS to contact the applicant before	cy on debt to the Federal Gove tee organization is responsible ingements are made with the a ire a grant can be made to con liquidate indebtedness to the F	ernment, the PHS shall (1) take such information into account whe e with respect to that grant, and (2) consider not making the gran agency to whom the debt is owed. Therefore, it may be necessa firm the status of the debt and ascertain the payment arrangements. Federal Government in a businesslike manner place themselves a
b. Debarment and Suspension.	Yes (If "Yes," attach	
department or agency. Subawardees, the	irment, declared ineligible, or vo lat is, other corporations, partn ant organization concerning the	tify, among other things, that neither it nor its principals are prese oluntarily excluded from covered transactions by any Federal nerships, or other legal entities (called "lower tier" participants), meir covered transactions. Please refer to the pertinent DHHS for complete certification requirements.
c. Drug-Free Workplace.	No (If "No," attach expla	·
certification require the applicant organization	ition to:	tify that it will provide a drug-free workplace. The main points of
 Publish a statement notifying employees substance is prohibited in the workplar Establish a drug-free awareness programmer. 	ice and specifying the actions	rre, distribution, dispensation, possession, or use of a controlled that will be taken against employees for violation of such prohibit
Require that each employee engaged is	in the performance of a grant of	or contract be provided a copy of the published statement;
		e will abide by the terms of the statement; drug violation occurring in the workplace; and
Require any employee who is convicted	ed of a drug offense occurring i	in the workplace to participate in a rehabilitation program.
requirements.	nertung regulations, Title 45 Co	ode of Federal Regulations Part 76, for complete certification
for-profit organizations, the rate established warrants, construction grants, grants to Federal	with the appropriate PHS Agend Lorganizations and grants to in	hed with the appropriate DHHS Regional Office, or, in the case of icy Cost Advisory Office. Indirect costs will not be paid on foreig ndividuals, and usually not on conference grants. Follow any Institutional National Research Service Awards, and specialized
X DHHS Agreement Dated:		No Indirect Costs Requested
No DHHS Agreement, but rates esta	ablished with	DATE
*CALCULATION Enter proposed budget period:		
Amount of Base \$ 140,097		% = Indirect Costs \$68,647
Add to total dire	ect costs from page 2 and ent	ter new total on FACE PAGE, Item 10b
*Check appropriate box(es)	□	
*Check appropriate box(es) Salary and wage base Off-site, other special rate, or more the	X Modified total direct co	

*This is the required last page of the application.